

TRIOSE COMPOUNDS IN MAPLE SIRUP^a

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The characteristic flavor and color of maple sirup are developed during the concentration of the maple sap by atmospheric boiling. An almost flavorless sirup has been produced by low-temperature vacuum evaporation of sap (9, 22). Further, by freeze-drying it is possible to make a completely colorless and flavorless product (22, 23). Therefore, the color and flavor of the sirup is formed by atmospheric boiling from normal constituents of maple sap.

The sap as it comes from the tree contains about 2% solids of which sucrose constitutes more than 98% (15). The remainder of the solids consists of organic acids (22), nitrogenous material (21), salts, and several oligosaccharides (24). Monosaccharides, if the sap remains sterile, are entirely absent. However, fermentation of the sap by contaminating microorganisms will cause inversion of the sucrose to amounts of invert sugar. Work at this laboratory (24), as well as that reported by Edson, Jones, and Carpenter (7), has shown that the growth of microorganisms in sap often results in dark-colored sirup. Hayward and Pederson (10) have added acids and alkalies to maple sap, and reported their effect on the sirup produced. During investigations on the color development in sirup (10, 31), it was discovered that the sap passed through an alkaline stage. With the earlier work as a background, the possibility of alkaline degradation of the hexoses was at once suggested as being part of the mechanism of color and flavor development in maple sirup. The alkaline degradation of sugars has been discussed by several authors. Evans in 1942 reported on the breakdown of glucose by alkalies (8). Reviews of color development (browning) in sugar systems have been published by Hodge (13) and Liggett and Deitz (17). Sattler and Zerban (26, 32) have discussed the chemical fragmentation of the unfermentable reducing substances in molasses. The ultraviolet spectra of sugars in alkaline solution have been studied by Berl and Feazel (2), who found a close relationship among such 3-carbon carbonyls (trioses) as acetol and reductone and the color chromogen of alkaline glucose solutions. Such compounds have been isolated from milk by steam distillation by Keeney and coworkers (16). Also, other carbonyl compounds related to flavor have been isolated by distillation from such diverse food products as cheese (5), corn oil (4), apples, (12, 14), strawberries (6), and pork (3).

Therefore it seemed possible that such carbonyl compounds as trioses could be isolated from U. S. grade AA sirup by steam distillation. Fancy grade maple sirup seemed the most likely source of carbonyl precursors

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as it is possible to cause a substantial increase in the color and flavor of this grade by the high-flavoring process (30). This paper presents evidence that trioses (3-carbon carbonyl compounds) are present in maple sirup and could be related to the alkaline breakdown of hexoses.

EXPERIMENTAL

Isolation of carbonyl compounds. A 200-g. sample of fancy grade maple sirup was steam distilled into 50 ml. of a solution containing 250 mg. of 2,4-dinitrophenylhydrazine^c (crystallized from benzene) first dissolved in 15 ml. of concentrated H₂SO₄. Three 200-ml. fractions were collected from the sirup. Two blanks were run, one before and one after the sample, using the same volume of water and steam. The 5 distillates were transferred to 250-ml. beakers, stirred, covered, and allowed to stand at 70°-75° F. overnight. The blanks did not contain 2,4 DNPH-precipitable material, but the first 200-ml. distillate contained a quantity of orange, amorphous precipitate. The other two distillates contained only a trace of precipitate and were discarded. The precipitate of distillate one was collected by filtration through a fritted glass disc by aspiration, washed with dilute sulfuric acid and a small quantity of distilled water, and dried in a vacuum at 50° C. Five-and-a-half milligrams of the dried mixture of 2,4 DNPH derivatives were obtained from the 200 g. of sirup.

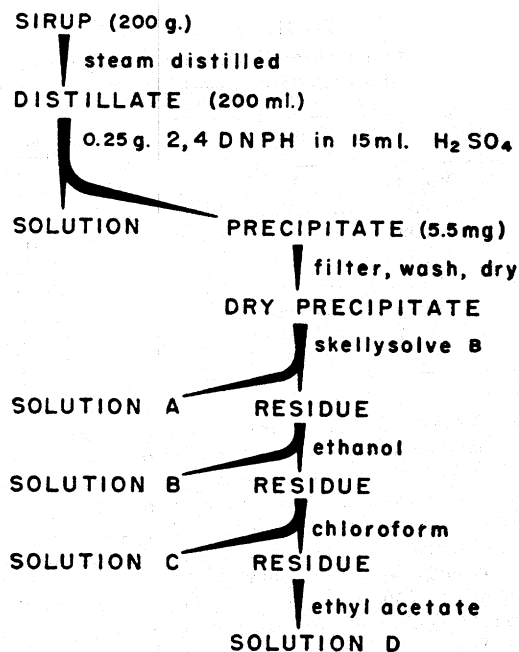
To determine roughly the type and amount of carbonyl compounds present, the dried mixture was treated with 10-ml. portions of petroleum ether (Skellysolve B,^d b.p. 63-70° C.), filtering off the solvent between each addition. This washing was repeated until the petroleum ether dissolved no more material. Then absolute ethanol was substituted for petroleum ether. Fractions dissolved by petroleum ether and by ethanol (solutions A and B) contained the monocarbonyl derivatives. The residue on the filter was then treated with chloroform to isolate the dicarbonyl derivatives (solution C). The small, final residue on the filter (Solution D) was soluble in ethyl acetate. While these solubility separations (Figure 1) furnished information as to the type of carbonyls present, it was later found that they served no particular purpose in the subsequent quantitative separation of the 2,4 DNPH derivatives in the original crude mixture.

Chromatographic separation of the 2,4 DNPH derivatives. After testing several types of chromatographic materials reported in the literature (4, 5, 6, 12, 25, 27, 29) for the columnar separation 2,4 DNPH derivatives, silicic acid was found best suited for separating the particular compounds isolated from the maple sirup. The crude precipitate was dissolved in chloroform and silicic acid was added, equivalent to about five times the weight of sample to form a slurry. This was evaporated to dryness at room temperature to precipitate the 2,4 DNPHs onto the acid. A silicic acid chromatographic column, 22 mm. x 15 cm., was prepared by adding a slurry of the material in petroleum ether (b.p. 63°-70° C.) to a tube 22 mm. ID x 40 cm. and packing it under two pounds of air pressure. The dry 2,4 DNPH-silicic acid mixture was transferred to the column with Skellysolve B. To effect a complete separation and elution of the derivatives, solvent mixtures were used starting with 5% diethyl ether in Skellysolve B. Several bands were formed from the orange mass at the top of the column. The polarity of the solvent was then raised by increasing the percentage of diethyl ether until 100% diethyl ether was being used. This was followed by a series of ethanol in diethyl ether in which the percentage of ethanol was increased from 25% to 100%. Finally the remaining bands were eluted with chloroform. From the elution of the developed bands 8 solvent fractions were obtained. The elution solvent, peak effluent volume, and color in alkaline ethanol are recorded for separated fractions in Table 1.

^c Hereafter designated as 2,4 DNPH.

^d Mention of specific commercial materials and equipment throughout this paper does not constitute recommendation for their use above similar materials and equipment of equal quality.

TRIOSE COMPOUNDS IN MAPLE SIRUP



SOLUTION A, B = MONOCARBONYLS
SOLUTION C = DICARBONYLS

Figure 1. Flow sheet of the solvent separation of the 2,4-dinitrophenylhydrazine derivatives of the steam distillable carbonyl compounds in maple sirup.

Identification of the 2,4 DNPH derivatives. A search of the literature failed to provide a very complete characterization of the 2,4 DNPH derivatives of carbonyl compounds of the type expected to be formed in maple sirup. Therefore, the 2,4 DNPH derivatives of a number of the carbonyl compounds related to possible alkaline sugar degradation products were prepared and carefully purified for the determination of such physical measurements as solubilities, melting points, spectral absorption curves and elemental chemical analyses. Details of the preparation, purification, and determination of the chemical and physical characteristics of these compounds will be described in another paper. Table 2 presents the physical data of a selected number of known compounds and those isolated from maple sirup. Only known compounds that have similarity to those obtained from maple sirup have been included.

TABLE 1
Data on the silicic acid column separation of 2,4 DNPH derivatives from maple sirup

Fraction	Eluting solvent	Peak effluent volume ml.	Color in alkaline methanol
1	10% diethyl ether in Skellysolve B	540-555	red
2	10% diethyl ether in Skellysolve B	710-800	red
3	15% diethyl ether in Skellysolve B	1650-1700	red
4	25% diethyl ether in Skellysolve B	1725-1765	red
5	100% diethyl ether	1780-2080	violet
6	50% diethyl ether in ethanol	2090-2140	violet
7	100% ethanol	2145-2205	violet
8	100% chloroform	2225-2500	violet

TABLE 2
Physical data on known and unknown 2,4-dinitrophenylhydrazine derivatives

Compound	Color in alkaline methanol	Melting point		Absorption solvent	Maxima λ
		Found ° C. (corr.)	Cited ° C.		
Acetol	Violet	295-298	296-97	(16) Dioxane	394,434
Methyl glyoxal	Violet	304-305	299-300	(11) Methanol	357,395
Glyceraldehyde	Violet	298-299	301(19)	Methanol	224,406,430
Reductone	Violet	Methanol	402,436
Hydroxymethyl furfural	Red	186	...	Methanol	384,299,262
Fraction 2	Red	104-106	...	Methanol	225,360
Fraction 5	Violet	177-185	...	Methanol	216,400
Fraction 8	Violet	296-299	...	Dioxane	394,434

Of the 8 fractions obtained from the chromatographic separation, only fractions 2, 5, and 8 contained sufficient material for the determination of their physical constants. These 3 isolates were evaporated to dryness at room temperature, and then completely dried at 50°C. in a vacuum oven. Weighed portions of the dry materials of fractions 2 and 5 were dissolved in dry methanol. Because of the low solubility of fraction 8 in methanol, 1,4-dioxane was used for this material to prepare a solution of sufficient concentration to make spectral photometric measurements. The absorption measurements from 225 $m\mu$ to 550 $m\mu$ were made on these 3 samples, using a Cary recording spectrophotometer. These measurements were compared with those of known 2,4 DNPH derivatives. The remaining materials of the 3 fractions were crystallized from acetone-water solution. Enough crystalline compound was obtained for melting point determinations. Data on the 3 fractions are listed in Table 2 along with similar data from known compounds.

RESULTS AND DISCUSSION

The largest portion of the steam distillable material from the maple sirup proved to be carbonyl compounds similar to one or more of the 3-carbon carbonyl compounds; glyceraldehyde, reductone, methyl glyoxal, and acetol. Fractions 5 and 8 from the chromatographic column consti-

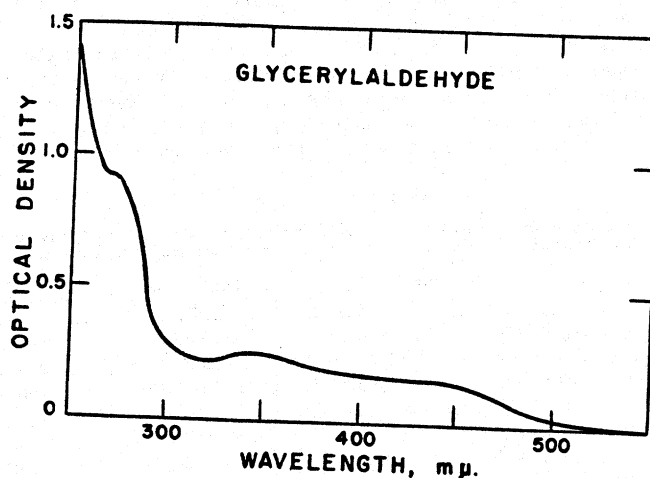


Figure 2. Absorption curve of the 2,4-dinitrophenylhydrazine derivative of glyceraldehyde in methanol.

TRIOSE COMPOUNDS IN MAPLE SIRUP

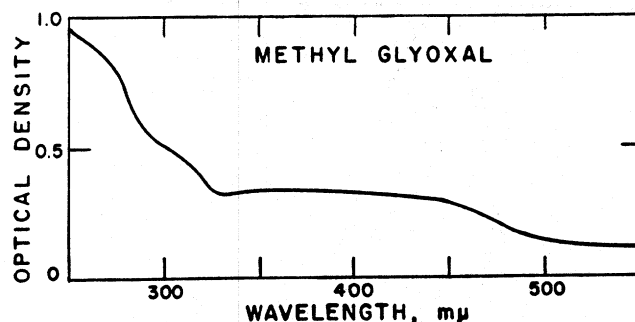


Figure 3. Absorption curve of the 2,4-dinitrophenylhydrazine derivative of methyl glyoxal in methanol.

tuted about 75% of the total amount of material in the steam distillate. Both fractions as shown by their violet color in alkaline methanol (19, 20, 27) as well as by their absorption curves (Figures 2, 3, 4, 5, and 6) were an osazone type of 2,4 DNPH derivative such as obtained from dicarbonyl and α -hydroxy carbonyl compounds (18, 28). The melting point of the crystalline compounds constituting fraction 8 corresponded to those obtained in this laboratory for the osazone 2,4 DNPH derivatives of purified methyl glyoxal, acetol, and glyceraldehyde and also agreed with the melting points cited in the literature for these compounds (1, 16, 19). No reference was found for the melting point of any 2,4 DNPH derivative of reductone, and the authors have yet to prepare a good crystalline 2,4 DNPH derivative of this compound. This is probably due to the many possible reaction products that could be obtained from a solution of reductone and 2,4 DNPH. However, the absorption curve of acetol in dioxane was almost identical with that of fraction 8 in the same solvent (Figure 7). The very low solubility of the acetol osazone in methanol required the use of dioxane as the solvent for its absorption curve. This was also true for

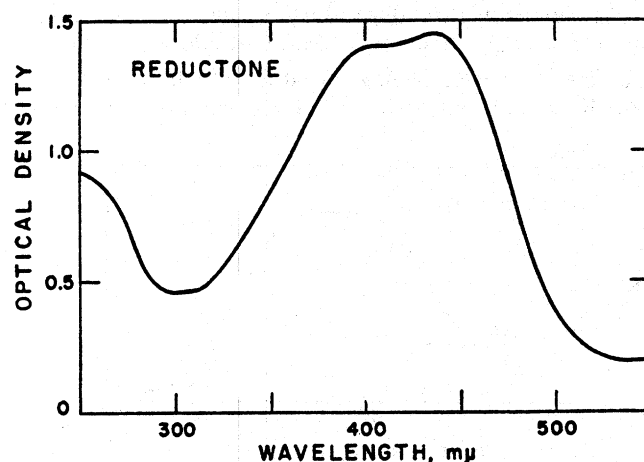


Figure 4. Absorption curve of the 2,4-dinitrophenylhydrazine derivative of reductone in methanol.

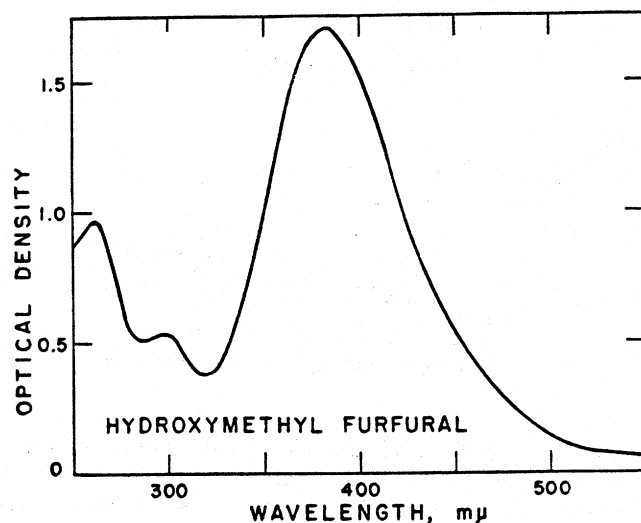


Figure 5. Absorption curve of the 2,4-dinitrophenylhydrazine derivative of hydroxymethylfurfural in methanol.

the material in fraction 8. This difference in the solubilities of the derivatives of the four 3-carbon carbonyls (trioses) favors the possibility that fraction 8 is acetol.

Regarding fraction 5, all of the physical data collectively indicate that this isolate was not a pure compound. Although the absorption curve of the fraction in methanol resembled the absorption curves of the osazones of reductone and glyceraldehyde in the same solvent, the melting point of the material crystallized from acetone-water was indefinite and much too low for the osazone of any of these compounds. However, the material of fraction 5 showed a closer relationship by its spectral absorption curve to the triose type compound than to other types of compounds sometimes involved in sugar and carbohydrate degradation such as the furfurals, pyruvic acid, and simple monocarbonyls as acetaldehyde or acetone.

Fraction 2 gave a few very well-formed crystals which melted at 104°-106° C. The alcoholic KOH test indicated a monocarbonyl derivative and

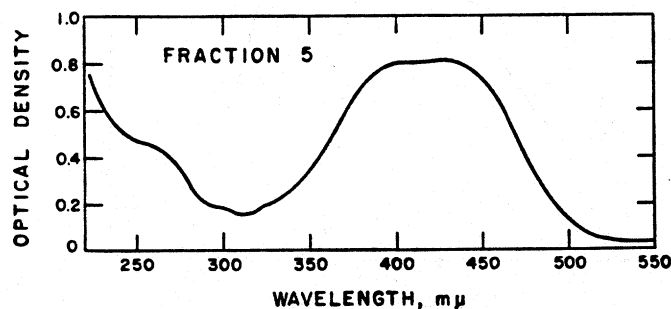


Figure 6. Absorption curve of the 2,4-dinitrophenylhydrazine derivative of fraction 5 in methanol.

TRIOSE COMPOUNDS IN MAPLE SIRUP

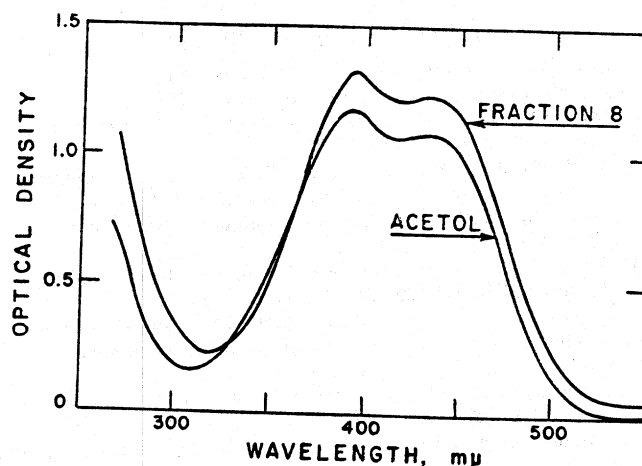


Figure 7. Absorption curves of the 2,4-dinitrophenylhydrazine derivatives of acetol and fraction 8 in dioxane.

the absorption curve with MeOH as solvent was also characteristic of a 2,4-dinitrophenylhydrazone. However, none of the 2,4 DNPH derivatives prepared by the authors from known carbonyls was found to compare closely with fraction 2. Examination of the literature showed that Allen (1) reported melting points for 2,4 DNPH derivatives of saturated, straight chain aldehydes C₇, C₁₀, and C₁₁ between 103° and 106° C. Buss and MacKinney (4) report the recovery, by steam distillation of rancid corn oil, of *n*-undecanal whose 2,4 DNPH derivative melted at 104° C. and whose absorption curve gave characteristics identical with fraction 2. Presence of this type of carbonyl compound in maple sirup was not foreseen theoretically. Its exact identity may furnish ideas for other mechanisms of flavor or color formation in maple sirup. Larger scale isolations of these carbonyl compounds by steam distillation and by several organic solvents are now under way.

Demonstration of the presence of triose carbonyl compounds in maple sirup suggests that they are intermediate in color and flavor development. These very reactive compounds through polymerization can form chromogens, and by interaction with other substances could form flavor bodies.

SUMMARY

Presence of trioses has been demonstrated in the steam distillate of maple sirup. The major portion of these compounds has been identified as probably acetol. These active carbonyl compounds are probable precursors of maple flavor and color.

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TRIOSE COMPOUNDS IN MAPLE SIRUP

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